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Molecular identification of *Mycobacterium tuberculosis* complex by region of differentiation-typing and polymerase chain reaction-restriction fragment length polymorphism method

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ABSTRACT

Tuberculosis (TB) is one of the most common zoonotic infectious diseases in the world. Identification of *Mycobacterium* isolates is essential for proper treatment of TB. The aim of this study was to identify *Mycobacterium* isolates collected from TB patients in Alborz Province, Iran, by region of differentiation (RD)-typing. Fifty samples from tuberculosis patients were cultured in pyruvate and glycerinated Lowenstein–Jensen medium. DNA was extracted from the isolates by the van Solingen method and subjected to polymerase chain reaction (PCR)-16SrRNA, PCR-IS6110, and RD-typing with primers RD1, RD4, RD9, and RD12, respectively. Out of 50 isolates, only one isolate appeared negative in IS6110-PCR and was considered nontuberculosis complex. The remaining isolates gave PCR products of approximately 543 bp, 245 bp, 146 bp, 172 bp, 235 bp, and 369 bp with 16s-rRNA, IS6110-PCR, RD-1, RD-4, RD-9, and RD-12 PCR, respectively. PCR-restriction fragment length polymorphism of *oxyR* pseudogene confirmed the results. All isolates except one from Alborz Province appeared positive for *Mycobacterium tuberculosis*. Based on the obtained results, all isolates except one were identified as *M. tuberculosis*. The only negative isolate appeared 93% and 97% similar to *Nocardia* or *Mycobacterium sp.* (*Mycobacterium neoaurum*), respectively, based on sequencing and alignment of 16s-rRNA and hsp65. Accurate identification of *Mycobacterium* isolates is of utmost importance for proper and immediate treatment of TB patients. In this study, RD-typing appeared to be a suitable method for correct identification of *M. tuberculosis* isolates.

Conflicts of interest

The authors declare no conflicts of interest.

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